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6. AUTHOR(S) Dr. Patricia A. Sobecky

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13. ABSTRACT (Maximum 200 words) Transfer of genetic information inter- and intragenetically determines the structure and function of indigenous bacterial communities. While plasmid exchange is an important mechanism by which bacterial populations can evolve and adapt, there remains a lack of information regarding the role of horizontal plasmid-mediated transfer in marine ecosystems and how indigenous broad-host-range plasmids impact the microbial community structure. In this study, the objectives will be to characterize plasmids, at the molecular level, from marine sediment bacterial populations to elucidate their role in promoting gene exchange. Specific objectives include: 1) determining the distribution and specific traits encoded by naturally occurring plasmids from marine sediment bacterial populations; and 2) assessing the mobility of indigenous broad-host-range plasmids in marine sediment microbial communities.	
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FINAL REPORT

GRANT #: N00014-98-1-0078

PRINCIPAL INVESTIGATOR: Dr. Patricia A. Sobecky

INSTITUTION: School of Biology
Georgia Institute of Technology
310 Ferst Drive
Atlanta, GA 30332-0230

Grant Title: Plasmid Diversity and Horizontal Transfer in
Marine Sediment Microbial Communities

AWARD PERIOD: 15 November 1997 - 30 September 2001

OBJECTIVE: The primary objective is to investigate and characterize the role of bacterial plasmids in facilitating microbial activities, interactions, adaptation and proliferation in marine environments. Toward this goal, studies are designed to gain an understanding of the effects of plasmid distribution, diversity and plasmid-mediated horizontal gene exchange on the structure and function of marine microbial communities.

APPROACH: To investigate plasmid distribution and plasmid-mediated effects on marine microbial community activities, plasmids are obtained from bacterial populations and characterized at the molecular level. DNA probes specific for replication regions (e.g., plasmid incompatibility-group probes) are used to characterize the distribution, diversity and persistence of the replicons in marine environments. Marine plasmids are also being sequenced to determine biological functions. The transfer dynamics of plasmids are assessed by elucidating environmental and molecular constraints likely to affect horizontal gene exchange. In addition, we are developing new molecular techniques to rapidly assess plasmid populations along spatial and temporal scales.

ACCOMPLISHMENTS: We have completed an extensive study using the collection of broad-host-range (BHR) marine plasmid origins as DNA probes to determine whether marine plasmids are geographically disseminated or constrained to 'local' niches. We have detected mobilizable and self-transmissible marine plasmids in different locales providing evidence that

BHR marine plasmids are widely disseminated. We have also identified a BHR plasmid that appears to be geographically constrained providing support for the local adaptation hypothesis that proposes that some plasmids encode traits that are adaptations to 'local' variations in environmental conditions. The plasmid (pPS172) is currently being sequenced at TIGR. A RAPD (randomly amplified polymorphic DNA) method suitable for use with marine plasmids has also been developed. The primer sequences have been designed for marine plasmids using sequence data obtained from TIGR. The PCR products are specific to the plasmid, and thus enable us to differentiate between plasmids. We are able to combine RAPD-PCR with our plasmid screening technique to determine the relatedness and therefore diversity of plasmids from sediment microbial communities. A collaboration with The Institute for Genomic Research (TIGR) to sequence as many as nine marine plasmids is currently in the annotation phase.

CONCLUSIONS: Our studies indicate that marine bacteria contain novel broad-host-range plasmids encoding unique replication regions. In addition, a PCR-based methodology we have developed for determining plasmid diversity provides evidence for considerable plasmid diversity in marine sediment microbial communities.

SIGNIFICANCE: Our findings have provided insights into the nature and diversity of plasmids in marine sediment bacteria. This information will aid in determining the role of mobile genetic elements in the adaptation of marine microbial communities.

PATENT INFORMATION: None

AWARD INFORMATION: The PI was an invited keynote speaker at the European Union (EU)-concerted symposium on "Mobile elements" contribution to bacterial adaptability and diversity (MECBAD) held in Prague, Czech Republic, September 15-19, 2000.

PUBLICATIONS AND ABSTRACTS (for total period of grant)

1. Cook, M.A., A.M. Osborn, J.A. Bettendorff, and P.A. Sobecky. 2001. Endogenous isolation of replicon probes for assessing plasmid ecology of marine sediment microbial communities. *Microbiology* 147:2089-2101.
2. Powers, L.G., J. Mallonee, and P.A. Sobecky. 2000. Nucleotide sequence and characterization of a cryptic

plasmid from a marine *Vibrio* sp. Plasmid 43:99-102.

3. Smalla K, E. Krogerrecklenfort, H. Heuer, W. Dejonghe, E. Top, M. Osborn, J. Niewint, C. Tebbe, M. Barr, M. Bailey, A. Greated, C. Thomas, S. Turner, P. Young, D. Nikolakopoulou, A. Karagouni, A. Wolters, J.D. van Elsas, K. Dronen, R. Sandaa, S. Borin, J. Brabhu, E. Grohmann and P. Sobecky. 2000. PCR-based detection of mobile genetic elements in total community DNA. *Microbiology* 46:1256-1257.
4. Reyes, N., M.E. Frischer, and P.A. Sobecky. 1999. Characterization of mercury resistance mechanisms in marine sediment microbial communities. *FEMS Microbiology Ecology*. 30:273-284.
5. Sobecky, P.A. 1999. Plasmid ecology of marine sediment microbial communities. *Hydrobiologia*. 401:9-18.
6. Sobecky, P.A., T.J. Mincer, M.C. Chang, A. Toukdarian, and D.R. Helinski. 1998. Isolation of broad-host-range replicons from marine sediment bacteria. *Appl. Environ. Microbiol.* 64:2822-2830.
7. Erdner, D.L.*, J. Eisen, and P.A. Sobecky. 2001. Whole plasmid sequencing and expression analysis of cryptic marine plasmids. American Soc. for Limnology and Oceanography Annual Meeting, Albuquerque, NM. Contributed. (*oral presentation).
8. M.A. Cook and P.A. Sobecky. May, 2000. Molecular characterization of plasmids in marine sediment microbial communities using replicon specific probes. American Soc. for Microbiol. Ann. Meeting Abstract.
9. Mills, H., M. Astwood, F.E. Loeffler, and P.A. Sobecky. May, 2000. Isolation and Characterization of plasmids from anaerobic marine sediment microbial communities. American Soc. for Microbiol. Ann. Meeting Abstract.
10. Powers, L.G., J.M. Mallonee, J.A. Bettendorff, C. Cain, P.A. Sobecky. May, 1999. Characterization of a Naturally Occurring Plasmid from a Marine *Vibrio* sp. for use in Assessing Horizontal Gene Transfer in Marine Microbial Communities. American Soc. for Microbiol. Ann. Meeting Abstract.
11. Reyes, N., M.E. Frischer, and P.A. Sobecky. May, 1999. Molecular characterization of marine microbial communities from PCB and Hg-contaminated salt marsh sediments. American Soc. for Microbiol. Ann. Meeting Abstract.

12. Sobecky, P.A.*, C.C. Cain, and M. Bellamy. February, 1999. Molecular Assessment of Plasmid Diversity in Marine Microbial Communities. American Soc. for Limnology and Oceanography Annual Meeting, Santa Fe, New Mexico. (*Invited oral presentation).
13. Sobecky, P.A.*, C.C. Cain, and T.J. Mincer. October, 1998. Unexpected Diversity in the Ocean: Plasmids isolated from marine bacteria contain replication and incompatibility regions unrelated to those of known plasmid groups. International Symposium on Plasmid Biology, Merida, Mexico. (*Invited oral presentation).
14. Cain, C.C., M.L. Bellamy, J.A. Bettendorff, and P.A. Sobecky. 1998. Molecular profiles of plasmids isolated from Georgia salt marsh sediment microbial communities. American Soc. for Microbiol. Ann. Meeting Abstract.